103(a) as being unpatentable over Prusiner *et al.* (U.S. Pat. No. 5,891,641), Martinez *et al.* (WO 96/05847), in view of Epps *et al.* (U.S. Pat. No. 6,203,994) or Kinjo *et al.* (*Nuc. Acids Res.*, 1995); and the rejection of claims 19-22 under 35 U.S.C. § 103(a) as being unpatentable over Eberwine *et al.* (WO 96/05847) or Epps *et al.* (U.S. Pat. No. 6,203,994), in view of Foster *et al.* (U.S. Pat. No. 4,444,879).

Claim Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 1-2, 4-8 and 10-14 are rejected under 35 U.S.C. § 112, second paragraph, 35 U.S.C. § 112, second paragraph, as being indefinite for failing to point out and distinctly claims the subject matter which Applicants regard as the invention.

Specifically, the Office Action states that claim 1 is vague and indefinite in reciting "detecting said protein by the binding of said labeled first binding partner **or** said labeled seecond binding partner" because it is unclear how detection is differentially effected based on which binding partner the protein binds to first.

Applicants have amended claims 1 and 5 to recite that a signal may be generated by the first and/or the second labeled binding partner, and that detection of the generated signal indicates the conformational state of the protein. Claim 13 depends from claims 1, and therefore incorporates the amendment of claim 1, claim 13 has also been amended to recite that the removal of unlabeled first binding partner is an additional step that occurs after step (c). Applicants and respectfully submit that these claims are clear on their face, and respectfully request that the rejection on this basis be reconsidered and withdrawn.

Claim 2 is also stated to be indefinite, the Office Action stating that it lacks a correlation step that structurally and functionally relates the binding of the protein with the first binding partner and the second binding partner with the post-translational modification activity of the enzyme so as to measure or determine post-translational modification activity of the enzyme as required by the preamble.

Applicants have amended this claim to add such a correlation step, namely, claim 2 has been amended to recite detecting the protein by the binding of at least one of the first and second labeled binding partner to the protein, and where detection of a signal indicates the activity of the enzyme. Applicants therefore respectfully request that the rejection on this basis be reconsidered and withdrawn.

Claim 5 is also rejected, the Office Action stating that this claim lacks antecedent basis in reciting, "said protein that does not bind to said capture ligand."

Applicants have amended this claim to provide such antecedent basis, and respectfully request that the rejection on this basis be reconsidered and withdrawn.

Claim 13 is also rejected, the Office Action stating that this claim is vague and indefinite in reciting, "unbound labeled first binding partner is removed" because it is unclear how "removal or separation" of unbound components is effected based on the limitations set forth in claims 1 or 2.

Applicants have amended this claim as well, and respectfully submit that as amended, the claim is clear on its face. Applicants respectfully request that the rejection on this basis be reconsidered and withdrawn.

Rejections Under 35 U.S.C. § 101 (Double Patenting)

Claims 1, 4-8, 10-14 and 19-22 stand provisionally rejected under 35 U.S.C. § 101 as claiming the same invention as that of claims 1-13 and 18-21 of copending U.S. App. No. 09/258,452. The Office Action states that the claims of U.S. App. No. 09/258,452 "do not appear to exclude that the binding partner or coupling of the protein with the first binding partner or the second binding partner is not covalent."

Because the subject matter claimed in these two applications is not identical, Applicants have not filed a Terminal Disclaimer at the present time. Upon an indication of allowable

subject matter in one or the other of the two applications, Applicants will revisit the issue of provisional double patenting, and will provide a Terminal Disclaimer.

Claim Rejections Under 35 U.S.C. §§ 102 and 103

The Office Action states that claims 1, 4-8 and 12-13 stand rejected under 35 U.S.C. § 102(e), as being anticipated by Prusiner *et al.* (U.S. Pat. No. 5,891,641) for reasons of record.

The Office Action also states that claims 1, 4-8 and 12-13 stand rejected under 35 U.S.C. § 102(e), as being anticipated by Martinez *et al.* (Int. App. WO 98/41872) for reasons of record.

The Office Action additionally states that claims 1, 7-8 and 10-11 stand rejected under 35 U.S.C. § 102(e), as being anticipated by Tsien *et al.* (U.S. Pat. No. 5,998, 204) for reasons of record.

The Office Action further states that claims 19-22 stand rejected under 35 U.S.C. § 103(a), as being unpatentable over Prusiner *et al.* (U.S. Pat. No. 5,891,641), Martinez *et al.* (Int. App. WO 98/41872), or Tsien *et al.* (U.S. Pat. No. 5,998, 204), in view of Foster *et al.* (U.S. Pat. No. 4,444,879), for reasons of record.

Claim Rejections in View of Prusiner et al.

Claims 1, 4-8 and 12-13 stand rejected under 35 U.S.C. § 102(e) as being anticipated by Prusiner *et al.* (U.S. Pat. No. 5,891,641), for reasons of record.

The previous Office Action (mailed June 7, 2001) states that Prusiner *et al.* discloses "a method for determining a diseased related conformational state of a protein such as PrP^{Sc} in a sample", and that this reference accomplishes this by "contacting the protein with a labeled antibody that binds (has higher binding affinity) to the protein in a manner dependent on the conformational state of the protein", that is, a diseased or a non-diseased state, and "contacting the protein with a second antibody or capture ligand to immobilize the protein on a solid phase substrate" (column 4, lines 5-10).

Applicants note that this characterization is not precisely correct. Rather, this and another section (column 1, line 65 to column 2, line 20) state that in the assay, the sample is

divided into two portions. The first portion is bound to a solid support and then labeled with an antibody that binds to the normal (*i.e.*, non-diseased) form of the protein. The antibody is such that it binds with a higher binding affinity to normal proteins rather than diseased proteins. The second portion of the sample is then treated to cause the diseased proteins to relax into a non-diseased conformation. This treated second portion of the sample is then also bound to a support substrate, and then treated with the same antibody. Whether or not proteins of the diseased form are in the original sample, and to what extent they are present, is determined by assaying the level of antibody binding in the first portion versus the second portion. That is, greater binding of the antibody in the second portion relative to the first portion indicates the existence in the sample of proteins in the diseased state form.

Prusiner *et al.* does not disclose "contacting the protein with a second antibody or capture ligand" as is indicated in the Office Action, nor is such a second antibody or capture ligand necessary "to immobilize the protein on a solid phase substrate".

Applicants' method, on the other hand, does not require dividing a sample into two portions as does that of Prusiner *et al.*, nor does it require treating one of the portions to alter the conformational state of the proteins in that portion prior to binding the proteins to the antibody. Applicants claims disclose determining the conformational state of a protein by binding a first and a second labeled binding partner to the protein. Prusiner *et al.* does not do this, nor does Prusiner *et al.*'s method come within the scope of Applicants' claims. Prusiner *et al.*'s method is simply an entirely different method.

Prusiner *et al.* describes what is essentially a "subtraction" method of detection, that is, the difference in antibody binding between the two portions is used to infer the presence of disease-state protein in the sample. Simple binding of the antibody of Prusiner *et al.* to the protein in the sample tells one nothing, because the antibody only binds to the protein when it is in a normal conformation. Although Prusiner *et al.* states that the antibody has a higher affinity for the normal protein relative to the disease protein, it is clear that the method would not work if the antibody bound the disease form to any appreciable degree. To detect the disease form of the protein, an aliquot of the sample must be manipulated and tested, relative to a control aliquot.

Strictly speaking, therefore, the method of Prusiner *et al.* does not actually detect the conformational state of a protein. The method instead uses binding level as a mathematical indication of probable conformational state. If the antibody used in the method fails to bind for some reason, then the method of Prusiner *et al.* will falsely indicate that the protein is in the diseased state.

Applicants' method does not require comparison to a control, but assays the protein's conformational state directly. Applicants submit that Prusiner *et al.* does not teach a method for detecting the disease-related conformational state of the PrP^{Sc} protein wherein the protein is detected by binding of a first and a second binding partner to the protein, as required by Applicants' claim 1. In contrast, Applicants' method detects the conformational state of a protein by detecting the binding of a first and a second binding partner to the protein, the conformational state of which is to be detected. The method of Prusiner *et al.* allows one to only infer indirectly the existence of the protein in a second (*i.e.*, disease-state) conformation as opposed to a first (*i.e.*, non-disease-state) conformation. Applicants' invention is therefore quite distinct from the teachings of Prusiner *et al.*, and Applicants therefore respectfully request that the rejection in view of this reference be reconsidered and withdrawn.

Claim Rejections in View of Martinez et al.

Claims 1, 4-8 and 12-13 stand rejected under 35 U.S.C. § 102(e) as being anticipated by Martinez *et al.* (WO 98/41872) for reasons of record. The previous Office Action (mailed June 7, 2001) states that this reference discloses "a method for determining a conformational state of a protein (activated versus inactivated) such as a cytokine receptor or growth hormone receptor (GHR) in a sample." The Office Action also states that this reference specifically discloses "contacting the protein with an antibody (GHR05) that selectively binds to the protein in a manner dependent on its conformational state, thereby forming a complex". The Office Action refers Applicants to the Abstract and also to page 1, lines 16-21 of this reference.

Applicants note that the Abstract states that the method determines cytokine receptor activation by "the use of an antibody capable of discriminating between an activated and an

inactive cytokine receptor conformation", by (i) "contacting the antibody and the cytokine receptor to form a complex", (ii) "contacting a candidate ligand to the complex", and (iii) "measuring the antibody binding to inactive cytokine receptor, . . . thereby discriminating between an activated and an inactivated conformation" of the receptor.

Applicants note that to anticipate a claim, a reference must disclose every element of the challenged claim and enable one skilled in the art to make the anticipating subject matter. This Martinez *et al.* does not do. This reference fails to disclose a first and a second labeled binding partner, both of which are recited in Applicants' claims. In addition, the monoclonal antibody disclosed in this reference (GHR05) binds to the human growth hormone receptor (hGHR) in both the absence or presence of human growth hormone (HGH). This is stated on page 4 of Martinez *et al.* where lines 22-23 state that the antibody binds to the receptor in the absence of human growth hormone, and in lines 11-12 of page 4, where it is stated that "[a]ntibody binding to the cell surface receptor increases upon receptor binding to growth hormone" (emphasis added). The antibody of Martinez *et al.* therefore does not bind to the protein "in a manner dependent on the conformational state of [the] protein", as is required by claim 1, but instead binds to the protein in both conformations. The reference does not explain how to differentiate between the two conformations when the antibody binds both.

Applicants also note that Martinez *et al.* is not prior art under 35 U.S.C. §102(b). As noted by the Examiner, this section of the law states that "the invention was patented or described in a printed publication . . . more than one year prior to the date of the application for patent in the United States." Applicants' filing date is February 26, 1999. To qualify as prior art under 102(b), Martinez *et al.* would have had to have been published before February 26, 1998. However, this reference was published September 24, 1998, and therefore fails to qualify as prior art under §102(b). The rejection on the basis of this reference must therefore be reconsidered and withdrawn.

Claim Rejections in View of Tsien et al.

The Office Action additionally states that claims 1, 7-8 and 10-11 stand rejected under 35 U.S.C. § 102(e), as being anticipated by Tsien *et al.* (U.S. Pat. No. 5,998, 204) for reasons of record. The previous Office Action (mailed June 7, 2001) states that this reference discloses "fluorescent indicators and methods for using them to determine concentration of binding partner (analyte) by determining a change in the conformational state of a protein". The Office Action also states that "the fluorescence indicators comprising a protein (binding protein) that changes conformation upon binding a first binding partner, wherein a first label (donor fluorescent moiety) is covalently bound to a binding protein moiety and a second label (acceptor fluorescent moiety) is bound to a binding protein moiety so that when the binding protein binds a binding partner, the fluorescent indicator is caused to change in conformation" (emphasis added).

Applicants note that the claimed invention does not cause a change in conformation of the protein, but instead detects a change in the conformation. As recited in claim 1, the labeled binding partner "[bind] to said protein in a manner dependent on the conformational state of said protein". (emphasis added). The claimed invention is therefore distinct from and not anticipated by Tsien *et al.*.

In addition, as pointed out in the Reply to the previous Office Action, the method of Tsien *et al.* requires that the donor fluorescent protein moiety and the acceptor fluorescent protein moiety be covalently coupled to the binding protein moiety (see, *e.g.*, col. 1, lines 48-64, and col. 4, lines 45-47), because a rigid attachment is necessary for the sensitive detection of ligand-induced conformational changes. As mentioned in the prior-filed reply, Tsien *et al.* states at column 5, paragraph 3 that

> If the GFP donor and acceptor are fused to a host protein rigidly, minor changes in the relative orientation of the ends of the latter would alter FRET. In contrast, most conventional fluorescent labels are attached by flexible linkers that at least partially decouple the fluorophore orientation from that of the protein to which its is attached, limiting the sensitivity of the FRET measurement.

All of Applicants claims either recite that the moieties are either "not covalently bound" (claims 1, 19 and 20), recite that each of such moieties "binds non-covalently" (claim 2), or depend from

such claims. Applicants therefore respectfully submit that Tsien *et al.* does not anticipate Applicants' claim, and respectfully request that the rejection on this basis be reconsidered and withdrawn.

Claim Rejections In View of Prusiner et al. or Martinez et al. or Tsien et al., and Foster et al.

The Office Action further states that claims 19-22 stand rejected under 35 U.S.C. § 103(a), as being unpatentable over Prusiner *et al.* (U.S. Pat. No. 5,891,641), Martinez *et al.* (Int. App. WO 98/41872), or Tsien *et al.* (U.S. Pat. No. 5,998, 204), in view of Foster *et al.* (U.S. Pat. No. 4,444,879), for reasons of record.

Prusiner *et al.*, Martinez *et al.* and Tsien *et al.* are discussed above. The Office Action relies on Foster *et al.* for the teaching of kit components with instructions for use in assay methods. The Office Action concludes that these four references render obvious the subject matter of Applicants' claims.

Applicants respectfully disagree. The Office Action has failed to make out a *prima facie* case of obviousness. The Manual of Patent Examining Procedure (MPEP) states at § 2142 that

> To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure.

(citing *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991)), and that

> The initial burden is on the examiner to provide some suggestion of the desirability of doing what the inventor has done. "To support the conclusion that the claimed invention is directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed invention or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references."

(citing *Ex parte Clapp*, 227 U.S.P.Q. 972, 973 (Bd. Pat. App. & Inter. 1985)).

As stated above, Prusiner *et al.* requires dividing a sample into two portions, treating one of the portions to alter the conformational state of the proteins in that portion, then binding the proteins to the antibody to see the differences between antibody binding in the two portions. The antibody described therein can only bind to a protein that is in a normal conformation, and Prusiner *et al.* therefore describes a "subtraction" method of detection, which can only infer the presence of diseased-state proteins indirectly.

Martinez *et al.* is not prior art under 35 U.S.C. § 102(b), and even if it were, discloses a monoclonal antibody (GHR05) that binds to the human growth hormone receptor (hGHR) in both the absence or presence of human growth hormone (HGH). It does not bind to the protein in a manner dependent on the conformational state of the protein, but instead binds to the protein in both conformations, and it is therefore not clear how one would differentiate between the two states.

Tsien *et al.* requires that the various binding moieties be covalently bound the the protein, so as to maintain a rigid association.

As stated above, none of these references, either alone or in combination, succeeds in providing the elements of Applicants' claims, let alone rendering the invention obvious. None of the references, either alone or in combination, teaches the binding of a first and a second labeled binding partner to a protein, where the labeled binding partners bind in a way that is dependent on the protein's conformational state (claim 1) or post-translational modification (claim 2). The addition of kits a lá Foster *et al.* do not supply the missing elements of Applicants' claims, and the Office Action therefore fails to establish a *prima facie* case of obviousness. Aplicajnts therefore request that the rejection on this basis be reconsidered and withdrawn.

Claim Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 1, 4-8, 10-14 and 19-22 are rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to

reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

Specifically, the Office Action states that the specification does not appear to provide any literal support for the recitation of "said protein and said labeled first binding partner or said second binding partner are not covalently coupled." As support for the rejection, the Office Action states that "[p]age 4, second full paragraph of the specification describes association and binding between protein and binding partners but fails to provide literal support for such recitation", and that the originally filed claims lacked such a limitation. The Office Action concludes that "[r]ecitation of claim limitation *[sic]* lacking literal support in the specification or originally filed claims constitutes new matter."

This conclusion is incorrect. There is no such requirement of literal support for claim limitations. The Manual of Patent Examining Procedure (MPEP) discusses the standard and procedure for determining compliance with the written description requirement. It states that "[a]n objective standard for determining compliance with the written description requirement is, 'does the description clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed.' " (MPEP § 2163.02, quoting *In re Gosteli*, 872 F.2d 1008, 1012, 10 U.S.P.Q.2d 1614, 1618 (Fed. Cir. 1989) The MPEP also states that in successfully showing possession of the invention, "[t]he subject matter of the claim need not be described literally (i.e., using the same terms or *in haec verba*) in order for the disclosure to satisfy the description requirement." (MPEP, § 2163.02, emphasis added).

Applicants have defined the terms "associates" and "binds" in the specification at page 4, lines 12-16:

> As used herein, the term "associates" or "binds" refers to binding partners as described herein having a binding constant sufficiently strong to allow detection of binding to the protein by a detection means. Preferably, the binding partners, when associated or bound, are in physical contact with each other and have a dissociation constant (Kd) of about 10mM or lower.

Related terms are also defined at page 4, lines 16-24:

> Therefore, the terms "substantially dissociated" and "dissociated" or "substantially unbound" or "unbound" refer to the absence or loss of contact

> between such regions, such that the binding constant is reduced by an amount which produces a discernible change in a signal compared to the bound state, including a total absence or loss of contact, such that the proteins are completely separated, as well as a partial absence or loss of contact, so that the body of the proteins are no longer in close proximity to each other but may still be tethered together or otherwise loosely attached, and thus have a dissociation constant greater than 10mM (Kd).

The discussion in the specification that binding partners have a disassociation constant, and that the terms "disassociated" and "unbound" can include "a total absence or loss of contact" and/or "a partial absence or loss of contact" is inconsistent with covalent binding between the protein and the binding partner. Applicants' recitation in claim 1 that a protein and a binding partner are not covalently bound simply recognizes what is stated in the specification, *i.e.*, that the protein and the binding partner can become unbound and lose contact, unlike components which are covalently bound.

It is well established that an applicant may be his own lexicographer, and that claim terms are to be construed in light of the specification. However, "no term may be given a meaning repugnant to the usual meaning of the term" (MPEP § 2173.05(a), quoting *In re Hill*, 161 F.2d 367, 73 U.S.P.Q. 482 (C.C.P.A. 1947)). Applicants respectfully submit that to interpret the discussion in the specification regarding dissociation as meaning that the protein and binding partners are covalently bound would be repugnant to the usual understanding of the term "dissociated".

As stated above, there is no requirement for literal recitation of claim terms in the specification. Applicants therefore respectfully submit that the phrase "not covalently coupled" is properly supported by the specification. However, Applicants have amended claim 1 to recite that the protein and binding partner are "not covalently bound". This phrase is also supported in the specification. In light of the support found in the specification, and the amendment to claim 1, Applicants respectfully request that the rejection on this basis be reconsidered and withdrawn.

Claim Rejections Under 35 U.S.C. § 103

Claims 2 and 14 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Prusiner *et al.* (U.S. Pat. No. 5,891,641), Martinez *et al.* (Int. App. WO 98/41872) and Tsien *et al.* (U.S. Pat. No. 5,998, 204), in view of Eberwine *et al.* (Int. App. WO 96/05847 and Epps *et al.* (U.S. Pat. No. 6,203,994) or Kinjo *et al.* (*Nuc. Acids Res.* 1995).

The Office Action concludes that it would have been obvious for one of ordinary skill in the art to apply the teachings of Eberwine *et al.* and Epps *et al.* in measuring post-translational modification activity of enzymes in the methods of Prusiner *et al.*, Martinez *et al.* and Tsien *et al.*, because enzyme activity is know to mediate conformational changes in proteins, and the disclosed methods of Prusiner *et al.*, Martinez *et al.* and Tsien *et al.* "are generic with respect to the type of protein being assayed for binding." The Office Action also states that it would have been obvious to one to substitute another known applicable detection method such as FCS as taught by Kinjo *et al.* and Epps *et al.* into the methods of Prusiner *et al.*, Martinez *et al.*, Tsien *et al.* and Eberwine *et al.*, because FRET or FCS constitute "an obvious design choice" for detecting binding bertween proteins.

The Prusiner *et al.*, Martinez *et al.* and Tsien *et al.* references are discussed above. In the previous Office Action, Eberwine *et al.* was characterized as disclosing a method for "measuring activity of an enzyme wherein the conformation of a protein is dependent upon the post-translational modification activity of the enzyme", including phosphorylation and glycosylation of the proteins. The Office Action also states that this reference discloses "contacting the protein which has a site (epitope) for post-translational modification with the enzyme." The Office Action also states that this reference further discloses "adding antibodies to bind at least two epitopes on the protein wherein one of the antibodies binds the protein in a manner dependent upon or specific for the post-translational modification of the protein by the enzyme."

Applicants note that page 4, line 10-34 and page 11, lines 3-33, which are cited by the Office Action, describe the method of Eberwine *et al.* as comprising "obtaining antibodies to at least two epitopes on a protein" (page 4, lines 12-13), and that "[t]hese antibodies are then

modified by binding a first nucleotide sequence and a second nucleotide sequence to said antibody" (page 4, lines 15-17). It then appears that two nucleotide sequences are "modified by binding a first nucleotide sequence and a second nucleotide sequence to said antibody". The two nucleotide sequences are described as being the same (see, *e.g.*, page 4, lines 21-24: "said nucleotide sequence comprising the same modified 5'-nucleotide . . . ."). The nucleotide sequences are hybridized together, and used to prime a double-stranded DNA synthesis. The double-stranded DNA is then digested, and the fragments separated and analyzed. It is not clearly stated how one would be able to differentially detect whether or not a protein was modified, because even though there were two different antibodies to attach to the two different types of proteins, the detection method will produce the same signal in either conformation. The only way in which the method of Eberwine *et al.* could actually function is if there were a different antibody created for each different conformation that one wished to detect, with a different pair of nucleotide sequences attached to it, which in turn primed the synthesis of a different double-stranded duplex.

A stated in the Reply to the prior Office Action, Eberwine *et al.* teaches a method for identifying an amino acid sequence of a protein, and for characterizing post-translational events occurring on the protein by epitope ordering followed by restriction mapping. The method of Eberwine *et al.* does not use labeled antibodies but rather uses antibodies bound to nucleotide sequences. This reference therefore does not teach a method for measuring the post-translational modification activity of an enzyme, where the method includes contacting a protein with labeled first and second binding partners, which bind to the protein in a manner dependent on the post-translational modification of the protein, and which generate a signal in a manner dependent on the post-translational modification of the protein by the enzyme, as is recited in amended claim 2. Rather, Eberwine *et al.* teach contacting a protein with a first unlabeled antibody bound to a nucleotide sequence and a second unlabeled antibody bound to a nucleotide sequence.

Essentially, Eberwine *et al.* teaches a method that is simply the creation of different antibodies specific for various types of proteins, and a unique way of tagging the antibodies so that each can be differentiated from the others. None of the antibodies in Eberwine *et al.* is

capable, on its own, of binding to a protein in a way that reflects the antibody's ability to differentiate between conformational states of that protein, and then producing a signal that provides information regarding the conformational state of the protein. Instead, the antibodies of Eberwine *et al.* either bind to a protein, or they do not, and the number of conformational states of the protein dictates the number of different and specific antibodies that must be produced.

Eberwine *et al.* therefore does not teach all of the elements of claim 2 or dependent claim 14.

The Office Action also states that the "nucleotide sequences used to modify the antibodies can be incorporated into solid phase", referring Applicants to page 6, lines 33-35. Applicants note, however, that this section does not discuss binding to a solid substrate, but rather states that "[t]he nucleotide sequence used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis." This section is clearly referring to synthesis and production and nucleotide sequences themselves, not binding the sequences to a solid phase substrate.

Applicants also respectfully submit that the Eberwine *et al.* reference is not enabling. The Abstract states that the method is for characterizing post translational events occurring on a protein, but it is not clear how the method actually works. Page 4, lines 10-34 appear to provide the best description of the method, and state that the method uses two different types of antibodies, one of which can recognize a post translationally modified protein, while the other antibody recognizes an unmodified protein. "These antibodies are then modified by binding a first nucleotide sequence and a second nucleotide sequence to said antibody". It is therefore not clear if each type of antibody has two nucleotide sequences attached to it, or if one nucleotide sequence is attached to each antibody. In addition, the two nucleotide sequences are described as being the same (see, *e.g.*, page 4, lines 21-24: "said nucleotide sequence comprising the same modified 5'-nucleotide . . . ."). It is therefore not clear how one would be able to differentially detect whether or not a protein was modified, because even though there were two different antibodies to attach to the two different types of proteins, the detection method will produce the same signal in either conformation.

Regarding the Epps *et al.* reference (U.S. Pat. No. 6,203,994), the Office Action states that this reference discloses "measuring activity of an enzyme wherein the conformation of a protein is dependent upon the post-translational modification activity of the enzyme." Applicants are referred to the Abstract of this reference, which the Office Action reports as stating that the method is used to determine the amount of substrate that is phosphorylated or dephosphorylated during the course of an enzymatic reaction.

The Office Action goes on to state that this reference discloses "contacting the enzyme with a protein (amino acid) that is capable of being phosphorylated by the enzyme and a reporter molecule comprising a fluorescent label and a phosphorylated protein, and an antibody that selectively binds to the phosphorylated protein" (referring to column 2, lines 33-54 and column 4, lines 20-41 of the reference).

Applicants note that this characterization is not precisely correct. The enzyme, with a phosphorylated amino acid attached, is reacted with its substrate (usually a protein). During the reaction, the phosphate is transferred from the enzyme to an identical amino acid on the substrate. An antibody is then used to determine the location of the phosphorylated amino acid -- on the substrate or on the enzyme -- to determine whether or not phosphorylation occurred, and to what extent. Epps *et al.* therefore discloses contacting a substrate with a labeled enzyme that is capable of transferring its label to the substrate during a phosphorylation reaction. Applicants note that the statement in the Office Action that the enzyme is contacted "with a protein (amino acid)" is incorrect. An amino acid is not a protein, and instead, the enzyme, with the amino acid attached, binds to the substrate protein, to which the amino acid is transferred.

Applicants' claims, in contrast, recite "contacting a protein with a labeled first binding partner" and also "a labeled second binding partner", where both binding partners bind to the protein "in a manner dependent on the conformational state of [the] protein", and where the first binding partner "generates a signal in a manner dependent on the binding of the first binding partner to the protein", and where the labeled second binding partner "can generate a signal in a manner dependent on the binding of the first binding partner to the protein". Epps *et al.* does not

disclose a binding partner capable of generating a signal, but a tagged enzyme capable of transferring the tag, which is then capable of being detected by an antibody. Furthermore, the antibody of Epps *et al.* binds the phosphorylated amino acid no matter where it is located (*i.e.*, on the enzyme before phosphorylation or on the substrate after phosphorylation). This reference therefore fails to disclose a binding partner which binds to a protein in a manner dependent on the protein's conformational state.

The Office Action states that Kinjo *et al.* teaches using fluorescence correlation spectroscopy (FCS) in monitoring the conformational state of a protein, and that this reference teaches that interaction kinetics of a fluorescent ligand with a target can be measured by a correlation function which describes the translational diffusion of bound and free ligand.

The Office Action concludes that one of ordinary skill in the art at the time the invention was made to apply the teaching of Eberwine *et al.* and Epps *et al.* in measuring post-translational modification activity of enzymes in the methods of Prusiner *et al.*, Martinez *et al.* and Tsien *et al.*, because enzyme activity is known to mediate conformational changes in proteins and the disclosed methods of Prusiner *et al.*, Martinez *et al.* and Tsien *et al.* are "generic" with respect to the type of protein being assayed for binding, and that it would have been obvious to have substituted another known applicable detection assay, such as FCS or FRET as taught by Kinjo *et al.* and Epps *et al.*

As stated above, Prusiner *et al.* requires dividing a sample into two portions, treating one of the portions to alter the conformational state of the proteins in that portion, then binding the proteins to the antibody to see the differences between antibody binding in the two portions. The antibody described therein can only bind to a protein that is in a normal conformation, and Prusiner *et al.* therefore describes a "subtraction" method of detection, which can only infer the presence of diseased-state proteins indirectly.

Martinez *et al.* discloses a monoclonal antibody (GHR05) that binds to the human growth

hormone receptor (hGHR) in both the absence or presence of human growth hormone (HGH). It does not bind to the protein in a manner dependent on the conformational state of the protein, but instead binds to the protein in both conformations, and it is therefore not clear how one would differentiate between the two states.

Tsien *et al.* disclose fluorescent indicators and methods for using them to determine concentration of an analyte binding partner by determining a change in the conformational state of a protein. However, this reference requires covalent binding between the indicator and the analyte, and also causes a change in the conformation state of the protein, neither of which is required in the present invention.

Eberwine *et al.* discloses antibodies that are prepared against "at least two epitopes" on a protein with "at least one antibody being directed toward a post-translationally modified epitope" -- in other words, the method disclosed in this reference requires at least two binding partners to detect a single post-translational modification (one detecting the modification, and the other antibody detecting the normal state), whereas Applicants' method requires only one, because each binding partner itself generates a signal, which is dependent on the conformation of the protein.

None of these references, either alone or in combination, succeeds in providing the elements of Applicants' claims, let alone render the invention obvious. Applicants' claims recite "contacting a protein with a labeled first binding partner" and also "a labeled second binding partner", where both binding partners bind to the protein "in a manner dependent on the conformational state of [the] protein", and where the first binding partner "generates a signal in a manner dependent on the binding of the first binding partner to the protein", and where the labeled second binding partner "can generate a signal in a manner dependent on the binding of the first binding partner to the protein". The addition of FRET detection in Epps *et al.* and FCS detection in Kinjo *et al.* fails to add the missing elements. In addition, the mere availability of binding assays does not provide motivation to combine references, nor does it provide an expectation of success if the proposed combination is made. On the contrary, Kinjo *et al.* teaches FCS assays involving the hybridization of nucleic acids, whereas Epps *et al.* describes

assays in which FRET is used to measure the binding of an antibody to a phosphorylated amino acid. The assumed correlation between a post-translational modification of a protein by an enzyme and conformational changes of said protein is therefore not supported by the teaching of Epps *et al.* or Kinjo *et al.*

Because there is no suggestion, either in the references or in the art, to combine the cited references, and because the references, even if combined fail to produce Applicants' invention, the rejection under 35 U.S.C. § 103 based on these references must be reconsidered and withdrawn.

Response to Applicants' Arguments

The Office Action states that Applicants' arguments have been fully considered but are not persuasive.

As stated by Applicants above, Prusiner requires that any sample be divided into aliquots, and also that one of the aliquots be "relaxed" *i.e.*, denatured. In addition, there is no direct measurement of the conformational state of the protein by measurement of a signal from the binding parter, but an indirect, mathematically-derived indication of such conformational state.

The Office Action also states that Martinez *et al.* discloses contacting a protein with a labeled first antibody and a second, unlabeled antibody. Applicants note that both of Applicants' binding partners are labeled. In contrast, the antibodies of Martinez *et al.* are not labeled *per se*, but are attached to nucleotide sequences. In addition, as discussed above, the antibodies of Martinez *et al.* do not actually bind to the target protein depending on its conformational state, but rather, the text of this reference reveals that the antibody binds to the protein in both conformational states.

The Office Action states that Tsien *et al.* does not require covalent coupling, and that this reference actually teaches against covalent coupling, referring Applicants to column 5, lines 22-27. Applicants quote this section:

> The invention can have one or more of the following advantages. Ligand-induced conformational changes can be monitored by FRET if, for example, the amino and carboxy termini of the binding protein are fused to a donor and acceptor GFP.

> This approach has several advantages over the usual covalent labeling with fluorescent probes.

This section of Tsien *et al.* is describing a fusion between the binding protein, and a donor and acceptor Green Fluorescent Protein, that is, it describes a fusion protein. This section of Tsien *et al.* is therefore stating that instead of covalently binding the various compoenents of the reaction in a test tube, it may be advantageous to have them welded into a fusion protein *in vivo*. A fusion protein, or any protein for that matter, can be described as a collection of amino acids that are covalently bound together in a chain. This section therefore does not represent a "teaching away" from covalent binding, but rather, increased support for the necessity of such binding in the method of Tsien *et al.*

The references of Prusiner *et al.*, Martinez *et al.* and Tsien *et al.* therefore cannot be relied upon, either alone or in combination, for teaching the subject matter of Applicants' claims.

Applicants submit that in view of the foregoing remarks, all issues relevant to patentability raised in the Office Action have been addressed. Applicants respectfully request the withdrawal of rejections over the claims of the present invention.

An extension of time and a Notice of Appeal from the Final Office Action dated November 27, 2001, are being filed concurrently, with appropriate fees. Please charge any deficiency or overpayment to Deposit Account No. 16-0085, Reference No. 10069/1170.

Respectfully submitted,

Date: May 28, 2002

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MARKED-UP VERSION OF AMENDMENTS:

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

Please amend claims 1-2, 5, 13, 19-20 and 22 as follows:

1. (Twice Amended) A method for determining the conformational state of a protein, comprising the steps of:

   a) contacting a protein with a labeled first binding partner which binds to said protein in a manner dependent on the conformational state of said protein and which generates a signal in a manner dependent on the binding of the first binding partner to the protein, and a labeled second binding partner which binds to said protein in a manner dependent on the conformational state of said protein and which can generate a signal in a manner dependent on the binding of the first binding partner to the protein, wherein said protein and said labeled first and [or] second binding partner are not covalently bound [coupled]; and

   b) detecting said protein by the binding of at least one of said labeled first binding partner or said labelled second binding partner to said protein wherein detection of a signal generated by said labeled first binding partner and/or said labeled second binding partner [labelling of said protein] is an indicator of the conformational state of said protein.

2. (Twice Amended) A method for measuring the post-translational modifying activity of an enzyme, wherein the conformation of a protein is dependent upon the post-translational modification activity of the enzyme, the method comprising the steps of:

a) contacting a protein comprising a site for post-translational modification with the enzyme;

b) providing a labelled first binding partner which binds non-covalently to the protein in a manner dependent on the post-translational modification of the protein by the enzyme and which generates a signal in a manner dependent on said post-translational modification, and a second labelled binding partner which binds non-covalently to said protein and which can generate a signal in a manner dependent on said post-translational modification;

c) contacting the protein with the labelled first binding partner and the labelled second binding partner and detecting said protein by the binding to said protein of at least one of said labeled first binding partner and said labeled second binding partner, wherein detection of a signal generated by said labeled second binding partner and/or said labeled second binding partner indicates [determining] the post-translational modifying activity of the enzyme.

5. (Twice Amended) The method of claim 1 or 2, wherein the second binding partner is a capture ligand, and said protein that binds to said capture ligand is isolated from a [said] protein that does not bind to said capture ligand.

13. (Twice Amended) The method of claim 1 or 2, further comprising the additional step of, after step (a), removing [wherein] unbound labelled first binding partner is removed to allow detection of the binding of the labelled first binding partner to the protein.

19. (Amended) A kit for the determination of the conformational state of a protein in a sample, comprising:

a) a labelled first binding partner which binds to the protein in a manner dependent on the conformational state of the protein and is detectable in a manner dependent on its binding to the protein and a labelled second binding partner, wherein said protein and said first binding partner or said second binding partner are not covalently bound [coupled]; and

b) packaging components.

20. (Amended) A kit for the determination of the presence of a ligand for a protein in a sample, comprising:

a) a protein which binds to the ligand the presence of which is to be determined and which undergoes a conformational change as a result of such binding;

b) a labelled first binding partner which binds to the protein in a manner dependent on the conformational state of the protein and is detectable in a manner dependent on its binding to the protein and a labelled second binding partner, wherein said protein and said first binding partner or said second binding partner are not covalently bound [coupled]; and

c) packaging components.

22. (Twice Amended) The kit of claim [clam] 21, wherein at least one of the first or second binding partner is labelled.